40 CFR Parts 795 and 799

[OPTS-42033C; FRL-2983-8(a)]

Cresols; Proposed Testing Standards

AGENCY: Environmental Protection Agency (EPA).

ACTION: Proposed rule.

SUMMARY: Elsewhere in this issue of the Federal Register, EPA is issuing a final test rule establishing testing requirements under section 4(a) of the Toxic Substances Control Act (TSCA) for manufacturers and processors of cresols. Cresols is a chemical category consisting of three cresol isomers: *ortho*-cresol (CAS No. 95-48-7), *meta*-cresol (CAS No. 108-39-4), and *para*-cresol (CAS No. 106-44-5). In this document, EPA is proposing that certain TSCA test guidelines be utilized as the test standards for the required studies. EPA is also proposing that tests be submitted within specified time frames.

DATES: Submit written comment on or before June 12, 1986. If persons request time for oral comment by May 28, 1986, EPA will hold a public meeting on this proposed rule in Washington, DC. For further information on arranging to speak at the meeting, see Unit VI of this preamble.

ADDRESS: Submit written comments, identified by the document control number (OPTS-42033C), in triplicate to: TSCA Public Information Office (TS-793), Office of Pesticides and Toxic Substances, Environmental Protection Agency, Rm. E-108, 401 M St., SW., Washington, DC 20460.

A public version of the administrative record supporting this action (with any confidential business information deleted) is available for inspection at the above address from 8 a.m. to 4 p.m., Monday through Friday, except legal holidays.

FOR FURTHER INFORMATION CONTACT: Edward A. Klein, Director, TSCA Assistance Office (TS-799), Office of Toxic Substances, Rm. E-543, 401 M St., SW., Washington, DC 20460. Toll Free: (800-424-9065), In Washington, DC: (554-1404), Outside the USA: (Operator 202-554-1404).

SUPPLEMENTARY INFORMATION: Elsewhere in this issue of the Federal Register, EPA is issuing a final test rule

under section 4(a) of TSCA to require testing of cresols for mutagenic effects, developmental toxicity, and reproductive effects. The Agency is proposing in this document the test standards to be used and the time frames for submission of the required test data.

I. Background

Elsewhere in this issue of the Federal Register, EPA is promulgating a Phase I final rule pursuant to TSCA section 4 that establishes testing requirements for manufacturers and processors of cresols. The Phase I rule specifies the following testing requirements for cresols: (1) Mutagenic effects studies (including tests for chromosomal aberrations, gene mutations, and cellular transformations) on specified cresol isomers; (2) developmental toxicity study with each cresol isomer; and (3) two-generation reproductive effects study with each cresol isomer.

Once this Phase I test rule becomes effective, manufacturers and processors of cresols would normally be required (under the two-phase test rule development process) to submit proposed study plans for each of these required studies and proposed schedules for both the initiation of testing and the submission of study data. (See 40 CFR 790.30, published in the Federal Register of May 17, 1985 (50 FR 20658).) EPA would review the submitted study plans and schedules and would thereafter issue them (with any necessary modifications) in a Phase II test rule proposal. This proposal would request public comment on the ability of the proposed study plans to ensure that the resulting data would be reliable and adequate. After evaluating and responding to public comment, EPA would adopt the study plans, including the reporting schedules, in a Phase II final rule as the required test standards and data submission deadlines. (See 40 CFR 790.32, published in the Federal Register of May 17, 1985 (50 FR 20659).)

However, in the case of the cresols test rule, which was initiated under the two-phase process, EPA has decided to propose the relevant TSCA test guidelines as the test standards (see Unit III below). In addition, EPA is proposing that the data from the required studies be submitted within certain time periods. These time periods will serve as the data submission deadlines required by TSCA section 4(b)(1) (see Unit IV below). The reasons for this change in the test rule development process for cresols are discussed below.

II. Change in the test rule development process

A. Test Standards and Data Submission Deadlines

TSCA section 4(b)(1) specifies that test rules shall include standards for the development of test data ("test standards") and deadlines for submission of test data. Under a two-phase test rule development process utilized by EPA since 1982 (47 FR 13012; March 26, 1982) and formally adopted in the fall of 1984 (49 FR 39774; October 10, 1984), test standards and data submission deadlines were to be adopted during the second phase of the rulemaking process. Upon issuance of the Phase I final rule, which established the effects and characteristics for which a given chemical substance must be tested, persons subject to the rule would be required by a specified date to submit study plans detailing the methodologies and protocols they intended to use to perform the required tests. Such study plans were to include proposed schedules for the initiation and completion of testing and submission of test data. (See 40 CFR 790.30 (a) and (c), published in the Federal Register of October 10, 1984 (49 FR 39774).) In the second phase, after consideration of public comment, the Agency would promulgate the Phase II final rule adopting the study plans (with any necessary modifications) as the test standards for the development of test data and deadlines for submission of test data.

In December 1983, the Natural Resources Defense Council (NRDC) and the Industrial Union Department of the American Federation of Labor-Congress of Industrial Organizations filed an action under TSCA section 20 which challenged, among other things, the use of the two-phase process. In an August 23, 1984 Opinion and Order, the Court found that utilization of the two-phase rulemaking process was permissible. However, the Court also held that the Agency was subject to a standard of promulgating test rules within a reasonable time frame. *NRDC* v. *EPA*, 595 F. Supp. 1255 (S.D.N.Y. 1984).

After the issuance of that Opinion, the Agency decided that to expedite development of section 4 test rules, it would utilize a single-phase rulemaking process for most test rules. In the document announcing this decision, EPA stated that the single-phase approach offers a number of advantages over the two-phase process (see 50 FR 20652, 20653; May 17, 1985). In this single-phase approach, the Agency proposes (in one document) not only the effects for which testing will be required, but also proposes pertinent TSCA test guidelines as the test standards and time frames for the submission of test data. After receiving and evaluating public comment on the proposed testing requirements, test guidelines, and data submission deadlines, EPA promulgates a final test rule.

This single-phase approach shortens the rulemaking period and expedites the initiation of required testing that would usually result from use of the two-phase rulemaking process. The single-phase process also eliminates the requirement under the two-phase approach for industry to submit test protocols for approval. Moreover, by allowing commenters to submit alternative testing methodologies during the comment period, the single-phase approach preserves the flexibility of the two-phase process.

These same advantages, i.e., expedited initiation of testing and the elimination of study plan submission requirements for persons subject to a Phase I rule, are factors considered by EPA in deciding to modify the rulemaking process for cresols. By proposing both pertinent TSCA test guidelines as the test standards and data submission deadlines at the time of issuance of the Phase I rule, EPA expects that the Phase II final rule will be issued 6 months sooner than would occur if the usual two-phase process was followed. Thus, required testing will be initiated on a more expedited basis. In addition, for each of the required tests for cresols, appropriate TSCA test guidelines are available (see Unit III below). Thus, EPA believes that there is no need for manufacturers and processors of cresols to develop study plans for approval independent of these TSCA guidelines.

B. Modifications to Requirements Under a Phase I Final Rule For Cresols

As indicated above, persons subject to the cresols Phase I final rule and who have notified EPA of their intent to test would normally be required to submit study plans and proposed data submission deadlines within a specified time of the final rule's effective date. (See 40 CFR 790.30(a) and (c), published in the Federal Register of May 17, 1985 (50 FR 20658).) However, because EPA is proposing certain TSCA test guidelines as the test standards and data submission deadlines, persons subject to the Phase I final rule are not required to submit proposed study plans for the required testing or proposed dates for the initiation and completion of that testing.

However, persons subject to the Phase I final rule for cresols are still

required to submit notices of intent to test or exemption applications in accordance with 40 CFR 790.25, published in the Federal Register of May 17, 1985 (50 FR 20657). Moreover, once the test standards are promulgated in the Phase II final rule, those persons who have notified EPA of their intent to test must submit study plans (which adhere to the promulgated test standards) no later than 30 days before the initiation of each required test.

III. Proposed Test Standards

The Phase I rule specifies that cresols be tested for mutagenic effects (including tests for chromosomal aberrations, gene mutations, and cellular transformations), development toxicity, and reproductive effects. The Agency is now proposing that this testing of cresols be conducted in accordance with specific guidelines set forth in Title 40 of the Code of Federal Regulations (CFR) as enumerated below. Test methods under new Parts 796, 797, and 798 were published in the Federal Register of September 27, 1985 (50 FR 39252). The health effects tests to be conducted are:

1. *Mutagenicity*: Chromosomal effects.
2. *In Vitro* Mammalian Cytogenetics test, which appears at 40 CFR 798.5375.
b. *In Vivo* Mammalian Bone Marrow Cytogenetics Tests: Chromosomal Analysis, which appears at 40 CFR 798.5385.
c. Rodent Dominant Lethal Assay, which appears at 40 CFR 798.5450.
2. *Mutagenicity*: Unscheduled DNA Synthesis in Mammalian Cells in Culture assay, which appears at 40 CFR 798.5550.
3. *Mutagenicity*: Gene mutations.
a. Detection of Gene Mutations in Somatic Cells in Culture assay, which appears at 40 CFR 798.5300.
b. Sex-linked Recessive Lethal Test in *Drosophila melanogaster*, which appears at 40 CFR 798.5275.
4. *Mutagenicity*: Cellular transformations. Morphologic Transformation of Mammalian Cells in Culture assay, which appears at 40 CFR 795.285.
5. *Developmental toxicity*: Developmental Toxicity Study, which appears at 40 CFR 798.4900.
6. *Reproductive effects*: Reproduction and Fertility Effects study, which appears at 40 CFR 798.4700.

EPA believes that the TSCA Health Effects Test Guidelines cited above, if properly followed, should produce adequate and reliable data. These guidelines describe methods for performing testing of chemical substance under TSCA. EPA reviews its TSCA test guidelines annually (see 47 FR 41857; September 22, 1982).

EPA has proposed in a separate Federal Register document (51 FR 1552; January 14, 1986), certain revisions to these TSCA guidelines to provide more explicit guidance on the necessary minimum elements for each study. These revisions will avoid repetitive chemical-by-chemical changes to the guidelines in their adoption as test standards for chemical-specific test rules. EPA is proposing that these modifications be adopted in the test standards for cresols.

The Agency believes that the TSCA test guidelines will provide relevant data to assess the potential human hazard resulting from exposure to cresols.

The chemical or physical properties, previous testing, specific manufacturing, use, disposal practices, and exposure patterns of a particular chemical, in some cases, will cause the Agency to make changes in TSCA guidelines which reflect the characteristics of a specific chemical. The changes made for the cresols in the guidelines and the justification for the use of these guidelines and for any changes are set forth below.

1. *In vitro mammalian cytogenetics test.* The *in vitro* cytogenetics test will detect structural chromosomal aberrations in cultured mammalian cells. For cresols, the solvent for the assay shall be dimethyl sulfoxide (DMSO) which was the solvent used for tesing the cresol isomers in previously conducted assays. In addition, the metabolic activation system for the assay shall be derived from Aroclor-1254 induced rat liver S-9 preparations because it is the system with the largest historical data base for use in this assay. Finally, the *in vitro* cytogenetic assay shall be performed in established cell lines or strains because of the historical data base available for these cell systems.

2. *In vivo mammalian bone marrow cytogenetics test.* The *in vivo* cytogenetics test is designed to detect structural chromosomal aberrations. For the use of cresols in this assay, the route of administration of the test substances shall be oral gavage. Furthermore, the test species shall be mice for the *in vivo* assay in order to allow species consistency among other required mutagenicity assays, i.e., rodent dominant lethal test, and also among upper-tier assays, i.e., heritable translocation assay, should they be required in the future.

3. *Rodent dominant lethal assay.* The rodent dominant lethal assay will determine whether or not a dominant lethal event is caused after exposure to a chemical substance. This dominant lethal event indicates that the test substance has affected germinal tissue of the test species. For cresols, the test substances shall be administered by oral gavage. Three dose levels shall be used. In addition, the test species shall be mice. Each male shall be mated to no more than two, and preferably to only one, female per mating interval. This is the optimum for this species. Further, females shall be left with the males for no longer than seven days, and mating shall continue for at least six weeks to ensure that the entire germ cell cycle is sampled.

4. *Unscheduled DNA synthesis in mammalian cells in culture.* This assay measures the repair of DNA damage induced by the test chemical. This assay shall be performed in primary cultures of rate hepatocytes because of the positive results obtained in this system with the equimixture of the isomers. In addition, the solvent used in the DNA damage assay shall be DMSO.

5. *Detection of gene mutations in somatic cells in culture.* This assay is used to detect possible mutations in mammalian cell culture systems induced by the chemical test substance. For cresols, the solvent used in this assay shall be DMSO. The metabolic activation system shall be derived from Aroclor-1254 induced rate liver S-9 preparations because it is the system with the largest historical data base for use in this assay. In addition, this assay shall be done in L5178Y cells because of a previous positive result in that cell system with the equimixture of the cresol isomers. Finally, a 4-hour exposure time shall be used because it is the standard exposure period recommended for this test.

6. *Sex-linked recessive lethal test in Drosophila melanogaster.* The sex-linked recessive lethal test using *Drosophila melanogaster* is designed to detect the occurrence of mutations, both point mutations and small deletions, in the germ line of the insect. For cresols, the oral route of administration shall be used in this assay. A concurrent negative control shall be included to ensure that any observed effects are a direct result of the chemical treatment. In addition, the results shall be confirmed in an independent assay because of the crucial nature of the results in determining any upper-tier mutagenicity testing in a subsequent test rule for cresols.

7. *Morphologic transformation of mammalian cells in culture.* This *in vitro* cellular transformation assay is a semi-quantitative assay for detection of the ability of chemical agents to morphologically alter cells in culture. Such transformation is associated with certain phenotypic changes such as loss of contact inhibition and the ability to form colonies in soft agar medium. The process by which these changes occur is

assumed to be closely related to the process of *in vivo* carcinogenesis. For cresols, *meta*-and *para*-cresol shall initially be tested in the cellular transformation assay performed without metabolic activation. *meta*- and *para*-Cresol shall be tested in the cellular transformation assay performed with metabolic activation only if they produce negative results in the cellular transformation assay without metabolic activation. *ortho*-Cresol shall only be tested in the cellular transformation assay performed with metabolic activation. This isomer has had negative results in a previously conducted cellular transformation assay without metabolic activation. Therefore, the next sequenced test is the cellular transformation assay with metabolic activation.

8. *Developmental toxicity study.* The developmental toxicity study is designed to provide information on the potential hazard to the unborn which may arise from exposure of the mother to the chemical test substance during pregnancy. Because of the large production and predicted widespread exposure of the human population to cresols, it was determined that cresols shall be tested for this effect. For cresols, the test substances shall be administered by oral gavage. In addition, the testing shall be performed in at least two mammalian species.

9. *Reproduction and fertility effects.* This guideline is designed to provide information concerning the effects of cresols on gonadal function, conception, parturition, and the growth and development of the offspring. The study can also give information about the effects of neonatal morbidity and mortality. It was determined that this test be required for cresols because of its substantial production, widespread use practices, and potential for widespread human exposure. For cresols, the test substance shall be administered by oral gavage.

IV. Reporting Requirements

EPA is proposing that all data developed under this rule be reported in accordance with its TSCA Good Laboratory Practice (GLP) standards, which appear in 40 CFR Part 792.

Further, test sponsors are required to submit individual study plans at least 30 days prior to beginning each study.

EPA is required by section 4(b)(1)(c) of TSCA to specify the time period during which persons subject to a test rule must submit test data. The Agency is proposing specific reporting requirements for each of the proposed test standards as follows:

1. The *in vitro* mammalian cytogenetics test shall be completed and final results submitted to the Agency within one year of the effective date of the final test rule.

2. The *in vivo* mammalian bone marrow cytogenetics test, if required, shall be completed and final results submitted to the Agency within one year of the effective date of the final test rule.

3. The rodent dominant lethal assay, if required, shall be completed and final results submitted to the Agency within two years of the effective date of the final test rule.

4. The unscheduled DNA synthesis in the mammalian cells in culture assay shall be completed and final results submitted to the Agency within one year of the effective date of the final test rule.

5. The detection of gene mutations in somatic cells in culture assay shall be completed and final results submitted to the Agency within one year of the effective date of the final test rule.

6. The sex-linked recessive lethal test in *Drosophila melanogaster*, if required, shall be completed and final results submitted to the Agency within two years of the effective date of the final test rule.

7. The morphologic transformation of mammalian cells in culture assay shall be completed and final results submitted to the Agency with one year of the effective date of the final test rule.

8. The development toxicity studies shall be completed and the final results submitted to the Agency within one year of the effective date of the final test rule.

9. The reproduction and fertility effects studies shall be completed and the final results submitted to the Agency within 29 months of the effective date of the final rule.

Interim progress reports shall be provided quarterly for each test. The progress reports shall begin 90 days after the effective date of the final Phase II test rule.

TSCA section 14(b) governs Agency disclosure of all test data submitted pursuant to section 4 of TSCA. Upon receipt of data required by this rule, the Agency will publish a notice of receipt within 15 days in the Federal Register as required by section 4(d). Test data received pursuant to this rule will be made available for public inspection by any person except in those cases where the Agency determines that confidential treatment must be accorded pursuant to section 14(b) of TSCA.

V. Issues for Comment

EPA invites comment on the use of the TSCA test guidelines and the chemical-specific modifications to these guidelines as the proposed test standards for the required testing of cresols. EPA also invites comment on the proposed schedule for the required testing.

VI. Public Meetings

If persons indicate to EPA that they wish to present oral comments on this proposed rule to EPA officials who are directly responsible for developing the rule and supporting analyses, EPA will hold a public meeting after the close of the public comment period in Washington, D.C. Persons who with to attend or to present comments at the meeting should call the TSCA Assistance Office (TAO): Toll Free: (800-424-9065); In Washington, DC: (544-1404); Outside the U.S.A. (Operator—202-544-1404), by May 28, 1986. A meeting will not be held if members of the public do not indicate that they wish to make oral presentations. While the meeting will be open to the public, active participation will be limited to those persons who arranged to present comments and to designated EPA participants. Attendees should call the TAO before making travel plans to verify whether a meeting will be held.

Should a meeting be held, the Agency will transcribe the meeting and include the written transcript in the public record. Participants are invited, but not required, to submit copies of their statements prior to or on the day of the meeting. All such written materials will become part of the EPA's record for this rulemaking.

VII. Public Record

EPA has established a record for this rulemaking [docket number (OPTS-42033C)]. This record includes basic information considered by the Agency in developing this proposal and appropriate Federal Register notices. The Agency will supplement the record with additional information as it is received.

This record includes the following information:

Supporting Documentation

(1) Federal Register notices pertaining to this proposed rule consisting of:

(a) Notice of final Phase I rule on cresols.

(b) Notice containing the ITC designation of cresols to the Priority List (42 FR 55026; October 12, 1977).

(c) Notice of proposed rule on cresols (48 FR 31812; July 11, 1983).

(d) Notice of final rule on EPA's TSCA Good Laboratory Practice Standards (48 FR 53922; November 29, 1984).

(e) Notice of final rule on test rule development and exemption procedures (49 FR 39774; October 10, 1984).

(f) Notice of final rule concerning data reimbursement (46 FR 41786; July 11, 1983).

(g) Notice of interim final rule on test rule development and exemption procedures (50 FR 20652; May 17, 1985).

(h) Notice of final rule on the C_9 Aromatic Hydrocarbon Fraction (50 FR 20662; May 17, 1985).

(i) Notice of final rule on mesityl oxide (50 FR 51857; December 20, 1985).

(2) Support documents consisting of:

(a) Cresols technical support document for proposed rule.

(b) Economic impact analysis of NPRM for cresols.

(c) Economic impact analysis of final test rule for cresols.

(3) Communications consisting of:

(a) Written public comments.

(b) Transcription of public meeting.

(c) Summaries of phone conversations.

(d) Meeting summaries.

(e) Reports—published and unpublished contractor's reports.

VIII. Other Regulatory Requirements

A. Executive Order 12291

Under Executive order 12291, EPA must judge whether a regulation is "Major" and therefore subject to the requirements of a Regulatory Impact Analysis. This test rule is not major because it does not meet any of the criteria set forth in section 1(b) of the Order. The economic analysis of the testing of cresols is discussed in the final test rule which appears elsewhere in this issue of the Federal Register.

B. Regulatory Flexibility Act

Under the Regulatory Flexibility Act (15 U.S.C. 601 *et seq.*, Pub. L. 96–354, September 19, 1980), EPA is certifying that this test rule, if promulgated, will not have significant impact on a substantial number of small businesses for the following reasons:

1. There is not a significant number of small businesses manufacturing cresols.

2. Small processors are not expected to perform testing themselves, or participate in the organization of the testing efforts.

3. Small processors will experience only very minor costs, if any, in securing exemption from testing requirements.

4. Small processors are unlikely to be affected by reimbursement requirements, and any testing costs passed on to small processors through price increases will be small.

C. Paperwork Reduction Act

The Office of Management and Budget (OMB) has approved the information collection requirements contained in the proposed rule under the provisions of the Paperwork Reduction Act of 1980, 44 U.S.C. 3501 *et seq.*, and has assigned OMB control number 2070–0033. Comments on these requirements should be submitted to the Office of Information and Regulatory Affairs of OMB, 726 Jackson Place, NW., Washington, DC 20503, marked "Attention . . . Desk Officer for EPA." The final rule package will respond to any OMB or public comments on the information collection requirements.

D. Comprehensive Environmental Response, Compensation and Liability Act ("Superfund")

The Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA) (42 U.S.C. 9601 *et seq.*, Pub. L. 96–510, December 10, 1980) requires that persons in charge of vessels or facilities from which hazardous substances have been released in quantities that are equal to or greater than the reportable quantities (RQs) immediately notify the National Response Center (NRC) of the release. (See CERCLA section 103(a), and 50 FR 13456; April 4, 1985.) The National Response Center can be notified at (800) 424–8802, except from the Washington, DC metropolitan area, where the telephone number for notification is (202) 426–2675. All designated hazardous substances will have an RQ of one pound until adjusted by regulation under CERCLA, unless such substances are already on the lost of CERCLA hazardous substances and have been assigned an RQ (see CERCLA section 102). Cresols have been assigned an RQ of 1,000 pounds.

List of Subjects in 40 CFR Parts 795 and 799

Testing, Environmental protection, Hazardous Substances, Chemicals, Recordkeeping and reporting requirements.

Dated: April 15, 1986.

John A. Moore,

Assistant Administrator for Pesticides and Toxic Substances.

Therefore, it is proposed that Subchapter R of Chapter I of Title 40 of the Code of Federal Regulations be amended as follows:

1. By adding Part 795, consisting at this time of § 795.285 under Subpart D, to read as follows:

PART 795—PROVISIONAL TEST GUIDELINES

Subparts A–C—[Reserved]

Subpart D—Provisional Health Effects Guidelines

Sec.

795.285 Morphologic transformation of cells in culture.

Authority: 15 U.S.C. 2603.

Subparts A–C—[Reserved]

Subpart D—Provisional Health Effects Guidelines

§ 795.285 Morphologic transformation of cells in culture.

(a) *Purpose.* *In vitro* assays for cellular transformation are semi-quantitative assays for the ability of chemical agents to morphologically alter (transform) cells in culture. Such transformation is associated with certain phenotypic changes such as loss of contact inhibition and the ability to form colonies in soft agar medium. The process by which these changes occur is assumed to be closely related to the process of *in vivo* carcinogenesis. Morphologically transformed cells appear as foci of dense, piled-up, altered cells on an underlying monolayer of normal cells. Three types of foci have been recognized. Type III foci appear to be most closely correlated with *in vivo* tumor formation. The ultimate criterion for morphologic transformation is the ability of the transformed cells to induce tumors when inoculated into appropriate hosts. Not all cells which appear to be morphologically transformed are capable of tumor formation. In general, there is reasonably good correlation between *in vitro* transformation and *in vivo* oncogenesis, although the correlation varies depending on the system being studied. These systems are believed to be reasonably good predictors of *in vivo* activity, and positive results are viewed as potential indications of *in vivo* carcinogenesis.

(b) *Definitions.* (1) Morphologic transformation is the acquisition of certain phenotypic characteristics most notably loss of contact inhibition and loss of anchorage dependence which are often but not always associated with the ability to induce tumors in appropriate hosts.

(2) Type III foci of transformed cells are multilayered aggregations of densely staining cells with random orientation and criss-cross arrays at the periphery of the aggregate. They appear as dark stained areas on a light staining background monolayer which is one-cell thick.

(c) *Reference substances.* Not applicable.

(d) *Test method*—(1) *Principle.* (i) Three systems for detecting chemically-induced morphologic transformation have been described. They are:

(A) Systems which employ cell lines (cells with an indefinite lifespan).

(B) Systems which employ cell strains (cells with a finite or limited lifespan).

(C) Systems which detect the interaction between chemicals and oncogenic viruses.

(ii) This study will employ an established cell line for detection of morphologic transformation.

(2) *Description.* Cells in culture are exposed to the test substance, both with and without metabolic activation, for a defined period of time. Cytotoxicity is determined by measuring the colony forming ability and growth rate of the cultures after the treatment period. At the end of the treatment period, cultures are maintained in growth medium for a sufficient period of time to allow near-optimal expression of tranformed foci.

(3) *Cells.* (i) Balb/c-3T3 mouse cells originally obtained from clone A-31 or its derivatives shall be used in the assay. Cells shall be checked for mycoplasm contamination prior to use in the assay and may be checked for karyotype.

(ii) Appropriate culture media and incubation conditions (culture vessels, CO_2 concentrations, temperature, and humidity) shall be used.

(4) *Metabolic activation.* Cells shall be exposed to test substance both in the presence and absence of a metabolic activation system. The metabolic activation system shall be derived from primary cultures of rat hepatocytes.

(5) *Control groups.* Positive and negative (untreated and vehicle) controls shall be included in each experiment. 3-Methylcholanthrene is an example of a positive control for experiments without metabolic activation. Dimethylnitrosamine is an example of a positive control in experiments with metabolic activation.

(6) *Test chemicals*—(i) *Vehicle.* Test agents shall be dissolved in serum-complete culture medium prior to treatment of the cells.

(ii) *Exposure concentrations.* Several concentrations (usually at least four) of the test substance shall be used. These shall be selected on the basis of a preliminary cytotoxicity assay performed both with and without metabolic activation. The highest concentration shall produce a low level of survival (approximately 10 to 20 percent) and the survival in the lowest concentration shall approximate that of the negative control.

(e) *Test performance.* (1) Cells shall be exposed to the test substance both with and without metabolic activation. Exposure shall be for 72 hours for experiments without metabolic activation and for 48 hours for experiments with metabolic activation unless different exposure times are justified by the investigator.

(2) At the end of the exposure period, cells shall be washed and cultured to determine viability and to allow for expression of transformation.

(3) At the end of the incubation period (generally four to six weeks), cells shall be fixed and stained, and the number of transformed (Type III) foci shall be enumerated.

(4) All results shall be confirmed in independent experiment.

(5) Tumorigenic potential of isolated morphologically transformed foci may be determined by inoculation into suitable hosts.

(f) *Data and report*—(1) *Treatment of results.* (i) Data shall be presented in tabular form. Individual colony counts for the treated and control groups shall be presented for both transformation and survival.

(ii) Survival and cloning efficiencies shall be given as a percentage of the controls. Transformation shall be expressed as a number of foci per dish, the number of dishes with transformed foci, and the transformed foci per number of surviving cells.

(2) *Statistical evaluation.* Data shall be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant concentration-related increase in the number of transformed foci. Another criterion may be based upon the detection of a reproducible and statistically significant positive response for at least one of the test substance concentrations.

(ii) A test substance which does not produce either a statistically significant concentration-related increase in the number of transformed foci or a statistically significant and reproducible positive response at any one of the test points is considered to be negative in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results for an *in vitro* mammalian cell transformation assay indicate that, under the test conditions, the test substance induces morphologic transformation in the cultured mammalian cells used.

(ii) Negative results indicate that, under the test conditions, the test substance does not induce morphologic transformation in the cultured mammalian cells used.

(5) *Test report.* In addition to the reporting recommendations as specified under Subpart J of 40 CFR Part 792, the following specific information shall be reported:

(i) Cell type used, including subclone designation and passage number; number of cell cultures; methods used for maintenance of cell cultures.

(ii) Rationale for selection of concentrations and number of cultures.

(iii) Test conditions: composition of media, CO_2 concentration, concentration of test substance, vehicle, incubation temperature, incubation time, duration of treatment, cell density during treatment, type of metabolic activation system, positive and negative controls, length of expression period (including number of cells seeded and subculture and feeding schedules, if appropriate).

(iv) Methods used to enumerate numbers of viable cells and transformed foci.

(v) Dose-response relationship, where possible.

(g) *References.* for additional background information on this test guideline, the following references should be consulted:

(1) Heidelberger, C., Freeman A.E., Pienta R.J., Sivak, A., Bertram, J.S., Casto, B.C., Dunkel, V.C., Francis, M.W., Kakunaga, T., Little, J.B., Schechtman, L.M. "Cell transformation by chemical agents—a review and analysis of the literature: a report of the U.S. Environmental Protection Agency Gene-Tox Program." *Mutation Research* 114:283–385, 1983.

(2) Kakunaga, T. "A quantitative system for assay of malignant transformation by carcinogens using a clone derived from Balb-3T3." *International Journal of Cancer* 12:463–473, 1973.

(3) Reznikoff, C.A., Bertram, J.S., Brankow, D.W., Heidelberger, C. "Quantitative and qualitative studies of chemical transformation of cloned C3H mouse embryo cells sensitive to post confluence inhibitions of cell division." *Cancer Research* 33:3239–3249, 1973.

(4) Reznikoff, C.A., Brankow, D.W., Heidelberger, C. "Establishment and characterization of a cloned line of C3H mouse embryo cells sensitive to post confluence inhibition of division." *Cancer research* 33:3231–3238, 1973.

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(6) Sivak, A., Tu, A.S. "Factors influencing neoplastic transformation by chemical carcinogens in Balb/c-3T3

cells." In: *The predictive value of short-term screening tests in carcinogenicity evaluation*. Williams, G.M., Kroes, R., Waaijers, H.W., Van de Poll, K.W., eds. Amsterdam, New York, Oxford: Elsevier/North Holland Biomedical Press, pp. 171–190, 1980.

(7) Williams, G.M. "Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell culture." *Cancer Research* 37:1845–1851, 1977.

PART 799—[AMENDED]

2. Part 799 is amended as follows:

a. The authority citation containues to read as follows:

Authority: 15 U.S.C. 2603, 2611, 2625.

b. In § 799.1250 by adding paragraphs (c)(1) (ii) and (iii), (2) (ii) and (iii), (3) (ii) and (iii), (4) (ii) and (iii), and (5) (ii) and (iii), to read as follows:

§ 799.1250 Cresols.

* * * * *

(c) * * *

(1) * * *

(ii) *Test standard.* (A)(*1*) In vitro mammalian cytogenetics test. This test shall be conducted with cresols in accordance with § 798.5375 of this chapter and modifications specified in paragraph (c)(l)(ii)(A)(*2*) of this section.

(*2*) Test standard modifications. The following modifications to § 798.5375 of this chapter are required.

(*i*) The requirement under § 798.5375 of this chapter is modified so that cresols shall be tested in established cell lines or strains. The cell line or strain used shall be checked for *Mycoplasm* contamination.

(*ii*) The requirement under § 798.5375 of this chapter is modified so that cresols shall be dissolved in DMSO prior to treatment of the cells.

(*iii*) The requirement under § 798.5375 of this chapter is modified so that the metabolic activation system for the assay shall be derived from Aroclor-1254 induced rat liver S-9 preparations.

(*iv*) The requirement under § 798.5375 of this chapter is modified so that at least three concentrations of the test substance over a range adequate to define the response shall be tested. The highest test concentration tested with and without metabolic activation shall be five milligrams per milliliter or that dose which show evidence of cytotoxicity or reduced mitotic activity.

(B)(*1*) In vivo mammalian bone marrow cytogenetics test. This chromosomal analysis test shall be conducted with cresols in accordance with § 798.5385 of this chapter and modifications specified in paragraph (c)(1)(ii)(B)(*2*) of this section.

(*2*) Test standard modifications. The following modifications to § 798.5385 of this chapter is modified so that the mouse is the required test species.

(*ii*) The requirement under § 798.5385 of this chapter is modified so that the test substance shall be administered once only by oral gavage.

(*iii*) The requirement under § 798.5385 of this chapter is modified so that three dose levels shall be used. The highest dose tested shall be the maximum tolerated dose or that producing some indication of cytotoxicity, e.g., partical inhabition of mitosis, or shall be the highest dose attainable.

(C)(*1*) *Rodent dominant-lethal assay.* This assay shall be conducted with cresols in accordance with § 798.5450 of this chapter and modifications specified in paragraph (c)(1)(ii)(C)(*2*) of this section.

(*2*) *Test standard modifications.* The following modifications to § 798.5450 of this chapter are required.

(*i*) The requirement under § 798.5450 of this chapter is modified so that the mouse is the required test species.

(*ii*) The requirement under § 798.5450 of this chapter is modified so that the route of administration of the test substance shall be by oral gavage.

(*iii*) The requirement under § 798.5450 of this chapter is modified so that three dose levels shall be used. The highest dose shall produce signs of toxicity, e.g., slightly reduced fertility, or shall be the highest dose attainable.

(*iv*) The requirement under § 798.5450 of this chapter is modified so that each male shall be mated to no more than two, and preferably to only one, female per mating interval. Females shall be left with the males for no longer than seven days, and mating shall continue for at least six weeks.

(iii) *Reporting requirement.* (A) The chromosomal aberration tests shall be completed and the final results submitted to the Agency as follows:

(*1*) The *in vitro* mammalian cytogenetics test within one year of the effective date of the final test rule.

(*2*) The *in vivo* mammalian bone marrow cytogenetics test, if required, within one year of the effective date of the final test rule.

(*3*) The rodent dominant lethal assay, if required, within two years of the final test rule.

(B) Interim progress reports shall be provided quarterly, beginning 90 days after the effective date of the final Phase II test rule.

(2) * * *

(ii) *Test standard.* (A) (*1*) *Unscheduled DNA synthesis in mammalian cells in culture assay.* This assay shall be conducted with cresols in accordance with § 798.5550 of this chapter and modifications specified in paragraph (c)(2)(ii)(A)(*2*) of this section.

(*2*) *Test standards modifications.* The following modifications ot § 798.5550 of this chapter are required.

(*i*) The requirement under § 798.5550 of this chapter is modified so that primary cultures of rat hepatocytes shall be the type of cells used in this assay.

(*ii*) The requirement under § 798.5550 of this chapter is modified so that cresols shall be dissolved in DMSO prior to treatment of the cells.

(B)(*1*) *Detection of gene mutations in somatic cells in culture.* This assay shall be conducted with cresols in accordance with § 798.5300 of this chapter and modifications specific in paragraph (c)(2)(ii)(B)(*2*) of this section.

(*2*) *Test standard modifications.* The following modifications to § 798.5300 of this chapter are required.

(*i*) The requirement under § 798.5300 of this chapter is modified so that cresols shall be tested in L5178Y mouse lymphoma cells. Cells shall be checked for *Mycoplasma* contamination.

(*ii*) The requirement under § 798.5300 of this chapter is modified so that cresols shall be dissolved in DMSO prior to treatment of the cells. The final concentration of the vehicle shall not interfere with cells viability or growth rate.

(*iii*) The requirement under § 798.5300 of this chapter is modified so that the metabolic activation system shall be derived from the postmitochondrial fraction (S–9) of rat livers pretreated with Aroclor 1254.

(*iv*) The requirement under § 798.5300 of this chapter is modified so that exposure shall be for four hours unless a different exposure time is justified by the investigator.

(C)(*1*) *Sex-linked recessive lethal test in Drosophila melanogaster.* This test shall be conducted with cresols in accordance with § 798.5275 of this chapter and modifications specified in paragraph (c)(2)(ii)(C)(*2*) of this section.

(*2*) *Test standard modifications.* The following modifications of § 798.5275 of this chapter are required.

(*i*) The requirement under § 798.5275 of this chapter is modified so that the oral route of administration shall be used in this assay.

(*ii*) The requirement under § 798.5275 of this chapter is modified so that a concurrent negative control shall be included in this assay.

(*iii*) The requirement under § 798.5275 of this chapter is modified so that the results of this test shall be confirmed in an independent assay.

(iii) *Reporting requirements.* (A) The gene mutation tests shall be completed and final results submitted to the Agency as follows:

(*1*) The unscheduled DNA synthesis in mammalian cells in culture assay within one year of the effective date of the final test rule.

(*2*) The detection of gene mutations in somatic cells in culture assay within one year of the effective date of the final test rule.

(*3*) The sex-linked recessive lethal test in *Drosophila melanogaster*, if required, within two years of the effective date of the final test rule.

(B) Interim progress reports shall be provided quarterly, beginning 90 days after the effective date of the final Phase II test rule.

(3) * * *

(ii) *Test standard.*—(A) *Morphologic transformation of mammalian cells in culture.* This test shall be conducted with cresols in accordance with § 795.285 of this chapter and modifications specified in paragraph (c)(3)(ii)(B) of this section.

(B) *Test standard modifications.* The following modifications of § 795.285 of this chapter are required.

(*1*) The requirement under § 795.285 of this chapter is modified so that *meta*- and *para*-cresol shall initially be tested in this assay performed without metabolic activation. Only if they produce negative results in the assay performed without activation will *meta*- and *para*-cresol then be tested in the assay with metabolic activation.

(*2*) The requirement under § 795.285 of this chapter is modified so that *ortho*-cresol shall only be tested in this assay performed with metabolic activation.

(iii) *Reporting requirements.* (A) The morphologic transformation of mammalian cells in culture assay shall be completed and final results submitted to the Agency within one year of the effective date of the final test rule.

(B) Interim progress reports shall be provided quarterly, beginning 90 days after the effective date of the final Phase II test rule.

(4) * * *

(ii) *Test standard.*—(A) *Developmental toxicity.* This study shall be conducted with cresols in accordance with § 798.4900 of this chapter and modifications specified in paragraph (c)(4)(ii)(B) of this section.

(B) *Test standard modifications.* The following modifications to § 798.4900 of this chapter are required.

(*1*) The requirement under § 798.4900 of this chapter is modified so that the test substance shall be administered by oral gavage.

(*2*) The requirement under § 798.4900 of this chapter is modified so that at least two mammalian species shall be used in this study.

(iii) *Reporting requirements.* (A) The developmental toxicity study shall be completed and final results submitted to the Agency within one year of the effective date of the final test rule.

(B) Interim progress reports shall be provided quarterly, beginning 90 days after the effective date of the final Phase II test rule.

(5) * * *

(ii) *Test standard.*—(A) *Reproduction and fertility effects.* This study shall be conducted with cresols in accordance with § 798.4700 of this chapter and modifications specified in paragraph (c)(5)(ii)(B) of this section

(B) *Test standard modifications.* The requirement under 798.4700 of this chapter is modified so that the test substance shall be administered by oral gavage.

(iii) *Reporting requirements.* (A) The reporduction and fertility effects study shall be completed and final results submitted to the Agency within 29 months of the effective date of the final test rule.

(B) Interim progress reports shall be provided quarterly, beginning 90 days after the effective date of the final Phase II test rule.

(Information collection requirements have been approved by the Office of Management and Budget under control number 2070–0033.)

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